Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
DEC 2008	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Release Of Bmp-2 And Tobramycin From Injectable, Biodegradable Polyurethane Scaffolds For Enhanced Bone Fracture Healing	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Vanderbilt University, Department of Chemical and Biomolecular Engineering and Center for Bone Biology, Nashville, TN**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES
See also ADM002187. Proceedings of the Army Science Conference (26th) Held in Orlando, Florida on 1-4 December 2008, The original document contains color images.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**UU**	**3**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

RELEASE OF BMP-2 AND TOBRAMYCIN FROM INJECTABLE, BIODEGRADABLE POLYURETHANE SCAFFOLDS FOR ENHANCED BONE FRACTURE HEALING

AE Hafeman[1,3], JO Hollinger*[2,3], SA Guelcher[1,3]
[1]Vanderbilt University, Department of Chemical and Biomolecular Engineering and Center for Bone Biology, Nashville, TN
[2]Carnegie Mellon University, Department of Biomedical Engineering, Pittsburgh, PA
[3]Center for Military Biomaterials Research, Piscataway, NJ

1. RELEVANCE TO THE ARMY

Nonunions are a significant clinical problem for civilians and military personnel. A 2004 report (*Capturing the Full Power of Biomaterials for Military Medicine*)[1] published by the National Research Council recognizes the potential for further development of bioactive materials that promote bone healing and decrease the incidence of non-unions. Military applications of bone graft materials incorporating bioactives that promote bone healing will be practical and realistic over the next 3 – 5 years. The report lists desirable characteristics and properties for a material to promote bone healing:

(1) Enhancement of the development of new blood vessels that serve to promote healing and preventing infections during bone and muscle repair.

(2) Ease of use (e.g., pastes and injectables that harden and cure *in vivo*).

(3) Biodegradability: degradation at a rate equal to that of bone healing *in vivo*.

(4) Bioactivity: controlled release of a wound-healing accelerant over a period of 1 to 3 weeks.

Infections are a significant clinical problem for civilians and military. Early reports indicate that approximately 65% of the wounds from the Iraq conflict culture positive for bacteria.[2] Open fractures are a common source of these infections, and represent the most challenging to treat. In a previous conflict, 4 of 11 open fractures became infected (3 of the 5 tibial fractures).[3] The soldiers who had complications, such as infection or non-union, had the longest hospital stay.

2. INTRODUCTION

A family of biocompatible, biodegradable polyurethanes (PUR) has been developed that degrade to non-toxic by-products and support cellular infiltration and new bone formation *in vivo*. In previous studies, biodegradable PURs have been shown to promote ingrowth of cells and new tissue formation *in vivo*.[4] Release of platelet-derived growth factor has also been shown[5], thus demonstrating the potential of these materials for controlled release of biologicals. These two-component materials are synthesized by reactive liquid molding, thus rendering them suitable for injection or casting into molds to form a desired shape. The clinical goal is to develop biologically active PUR and implantable and injectable biomaterials that promote bone wound healing and decrease the incidence of non-unions and infection.

3. MATERIALS AND METHODS

3.1. Synthesis and Characterization

PUR scaffolds were synthesized by reactive liquid molding of hexamethylene diisocyanate trimer (Bayer Materials Science LLC) (or lysine triisocyanate, LTI, Kyowa Hakko) and a hardener comprising a polyester triol, PEG600, water, catalyst, stabilizer, and pore opener using previously reported techniques [6]. Materials were characterized by dynamic mechanical analysis (DMA), differential scanning calorimetry (DSC), density (gravimetrically), and pore size (SEM). Degradation was evaluated by incubating the materials in PBS at 37^{o}C for up to 12 weeks and measuring the weight loss at each time point.

3.2. *In vitro* Release of Biologicals

Release experiments were performed by incubating the scaffolds in PBS at 37^{o}C for up to 4 weeks and measuring the concentration of the biological at specified time points. Lyophilized tobramycin (8 wt%) and bone morphogenetic protein (BMP-2) (0.001 wt%) with heparin (0.03 wt%) were incorporated separately into foams. [7, 8] *In vitro* release of tobramycin and BMP-2 in PBS at 37 ºC was measured from 0.5 to 28 days. BMP-2 release was measured by ELISA. The released tobramycin was quantified using a HPLC assay and evaluated for bioactivity with a Kirby-Bauer test after 24 hrs on methicillin-susceptible *S. aureus*. For time-kill experiments, *S. aureus* was inoculated into trypticase soy broth and incubated for 18 hrs at 37 °C, from which two dilutions were made: 10^2 and 10^7 CFU/mL. 200 mg of foam containing tobramycin was added to each solution.

200 µL aliquots of broth were removed at regular intervals from 0 to 24 days, and plated at four dilutions onto 5% sheep blood agar. These plates were incubated at 37 °C for 24 hours and then colony counts performed.

3.3. New Bone Formation *in vivo*

To evaluate the potential of the scaffolds to promote new bone formation *in vivo*, the materials were implanted in bilaterial, uni-cortical tibial plug defects in rats. Implants were harvested after 3 weeks, embedded in PMMA, sectioned for histology, and stained with toluidene blue.

4. RESULTS AND DISCUSSION

4.1. *In vitro* Release of BMP-2

Release of BMP-2, shown in Figure 1, is characterized by a burst release of ~60%, followed by a slower release up to 21d. After 21d, approximately 80 – 90% of the BMP-2 had been released. While a higher cumulative release was achieved when BMP-2 powder was incorporated in the scaffolds, incorporation of BMP-2 in PLGA microspheres prior to addition to the scaffolds resulted in a substantially lower burst release and more sustained release from day 1 – day 12. A more sustained release may enhance bone formation relative to a burst release *in vivo*. [9]

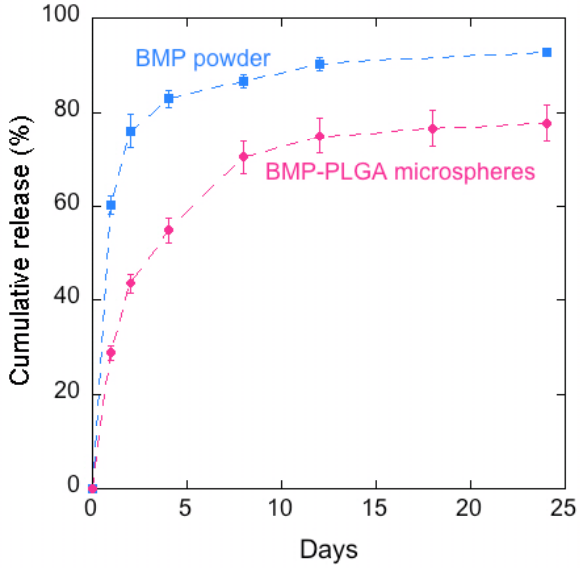

Figure 1. In vitro release of BMP-2. (a) incorporated as a lyophilized powder. (b) incorporated as encapsulated in PLGA microspheres.

4.2. *In vitro* Release of Tobramycin

In vitro release of tobramycin (data not shown) exhibited a similar profile as observed for BMP-2, characterized by a burst of approximately 40 – 80% on day 1. Kirby-Bauer tests revealed that sufficient tobramycin diffused from the foams and to inhibit the growth of bacteria (Figure 2). All ZI were larger than the minimum sensitivity levels of 15 mm and comparable to the positive controls (PMMA). Both time-kill dilutions yielded zero colonies through 24 days, confirming that the tobramycin released from the foams killed all the *S. aureus* present.

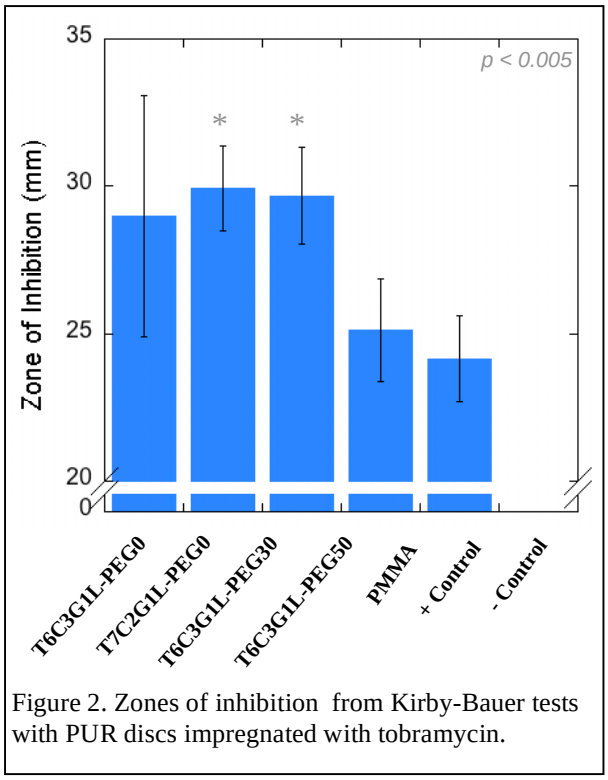

Figure 2. Zones of inhibition from Kirby-Bauer tests with PUR discs impregnated with tobramycin.

4.3. New bone formation *in vivo*

A representative histological section of a PUR implant is shown in Figure 3. Toluidene blue histology showed extensive cellular penetration into the implant (no BMP-2 or tobramycin) and new bone formation, as evidenced by the dark blue stained regions, by day 21. The wounds experienced little inflammation and there was no sign of an adverse immune response. These data demonstrate the potential of the PUR scaffolds without added growth factors to promote new bone formation throughout the implant as early as 3 weeks.

5. CONCLUSIONS

PUR scaffolds show promise as an effective strategy for bone fracture healing. Their elastomeric properties promote thorough contact with the surrounding bone, and the material properties can be tuned for varied

strength and elasticity. *In vivo* studies show extensive cellular infiltration and new tissue formation in soft tissue with minimal inflammation. Furthermore, we can release growth factors and antibiotics from these foams in a controlled manner. The materials can be processed by reactive liquid molding and are therefore suitable for injectable applications.

Figure 3. New bone formation in a rat tibia uni-cortical plug model.

6. ACKNOWLEDGEMENTS

This work was funded by the Orthopaedic Trauma Research Program (OTRP), the Armed Forces Institute for Regenerative Medicine, Vanderbilt University, and the Bone Tissue Engineering Center at Carnegie Mellon University.

7. REFERENCES

1. National Research Council. *Capturing the Full Power of Biomaterials for Military Medicine*; National Research Council: Washington, DC, 2004; p 16.
2. Doukas, W. C. In *Combat Orthopaedics: Ongoing Care in the Global War on Terror*, AAOS 2005 Annual Meeting, 2005; 2005.
3. Mabry, R. L. *J Trauma* **2000,** 49, (3), 515-528.
4. Gogolewski, S.; Gorna, K. *J Biomed Mater Res* **2007,** 80A, 94-101.
5. Hafeman, A.; Li, B.; Yoshii, T.; Zienkiewicz, K.; Davidson, J.; Guelcher, S. *Pharm Res* **2008,** In Press.
6. Guelcher, S.; Srinivasan, A.; Hafeman, A.; Gallagher, K.; Doctor, J.; Khetan, S.; McBride, S.; Hollinger, J. *Tissue Engineering* **2007,** 13, (9), 2321-2333.
7. Ambrose, C. G.; Gogola, G. R.; Clyburn, T. A.; Raymond, K. A.; Peng, A. S.; Mikos, A. G. *Clinical Orthopaedics & Related Research* **2003,** 415, 279-285.
8. Zhao, B.; Katagiri, T.; Toyoda, H.; Takada, T.; Yanai, T.; Fukuda, T.; Chung, U.-i.; Koike, T.; Takaoka, K.; Kamijo, R. *J. Biol. Chem.* **2006,** 281, (32), 23246-23253.
9. Seeherman, H.; Li, R.; Bouxsein, M.; Kim, H.; Li, X. J.; Smith-Adaline, E. A.; Aiolova, M.; Wozney, J. M. *J Bone Joint Surg* **2006,** 88-A, 144-160.